Shixia Feng get * items first please

Ketones or aldehydes and the formation of protein adducts

1: Biochemistry 2002 Sep 24;41(38):11466-71

Identification of oxidized derivatives of neuroketals.

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Oxidative stress and protein aggregation have been implicated in the pathogenesis of neurodegenerative diseases. The formation of neuroprostanes, isoprostane-like compounds formed from oxidation of docosahexaenoic acid, which is uniquely enriched in the brain, is increased in Alzheimer's disease. We recently identified the formation of a new class of highly reactive gamma-keto aldehydes, neuroketals, in vivo as products of the neuroprostane pathway. Neuroketals adduct to lysine residues of proteins with remarkable rapidity and induce cross-linking. Because neuroketals have either a 1,4-pentadiene or 1,4,7-octatriene side chain structure, we hypothesized that they could undergo further oxidation to form neuroketals with an additional hydroxyl group. Oxidation of docosahexaenoic acid in vitro yielded a series of compounds that were confirmed to be oxidized neuroketals by mass spectrometric analyses. Analysis of oxidized neuroketal adducts during oxidation of docosahexaenoic acid in the presence of lysine revealed the formation of oxidized Schiff base and hydroxylactam adducts. Oxidized hydroxylactam neuroketal-lysyl protein adducts, analyzed after digestion of proteins to individual amino acids, were not detected in nonoxidized rat brain synaptosomes but were readily detected following oxidation of synaptosomes. These studies indicate that neuroketals can undergo further oxidation, which in turn suggests that measurement of only unoxidized neuroketal adducts likely underestimates the amount of neuroketal adducts present in the brain in disorders of oxidant stress.

PMID: 12234189 [PubMed - indexed for MEDLINE]

2: Am J Clin Nutr 2002 Sep;76(3):668-74

Generation of protein adducts with malondialdehyde and acetaldehyde in muscles with predominantly type I or type II fibers in rats exposed to ethanol and the acetaldehyde dehydrogenase inhibitor cyanamide.

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BACKGROUND: Alcoholic myopathy is known to primarily affect type II muscle fibers (glycolytic, fast-twitch, anaerobic), whereas type I fibers (oxidative, slow-twitch, aerobic) are relatively protected. OBJECTIVE: We investigated whether aldehyde-derived adducts of proteins with malondialdehyde and acetaldehyde are formed in muscle of rats as a result of acute exposure to ethanol and acetaldehyde. The differences between type I muscle, type II muscle, and liver tissue were also assessed. DESIGN: The formation and distribution of malondialdehyde- and acetaldehyde-protein adducts were studied with immunohistochemistry in soleus (type I) muscle, plantaris (type II) muscle, and liver in 4 groups of rats. The different groups were administered saline (control), cyanamide (an acetaldehyde dehydrogenase inhibitor), ethanol, and cyanamide + ethanol. RESULTS: Treatment of rats with ethanol and cyanamide + ethanol increased the amount of aldehyde-derived protein adducts in both soleus and plantaris muscle. The greatest responses in malondialdehyde-protein and acetaldehyde-protein adducts were observed in plantaris muscle, in which the effect of alcohol was further potentiated by cyanamide pretreatment. Malondialdehyde- and acetaldehyde-protein adducts were also found in liver specimens from rats treated with ethanol and ethanol + cyanamide; the most abundant amounts were found in rats given cyanamide pretreatment. CONCLUSIONS: Acute ethanol administration increases protein adducts with malondialdehyde and acetaldehyde, primarily in type II muscle. This may be associated with the increased susceptibility of anaerobic muscle to alcohol toxicity. Higher acetaldehyde concentrations exacerbate adduct formation, especially in type II-predominant muscles. The present findings are relevant to studies on the pathogenesis of alcohol-induced myopathy.

PMID: 12198016 [PubMed - indexed for MEDLINE]

3: Am J Respir Crit Care Med 2002 Aug 15;166(4):490-5

4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease.

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Cigarette smoking results in oxidative stress and inflammation in the lungs, which are involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). 4-Hydroxy-2-nonenal (4-HNE), a highly reactive diffusible product of lipid peroxidation, is a key mediator of oxidant-induced cell signaling and apoptosis. 4-HNE has a high affinity toward cysteine, histidine, and lysine groups and forms direct protein adducts. We investigated the presence of 4-HNE-modified proteins in lung tissue obtained from subjects with and without COPD. We studied 23 current or ex-smokers with similar smoking histories with COPD (n = 11; FEV(1) < 70% predicted) or without COPD (n = 12; FEV(1) > 84% predicted) who had undergone lung resection. As 4-HNE and transforming growth factor-beta(1) (TGF-beta(1)) can modulate gamma-glutamylcysteine synthetase (gamma-GCS) mRNA levels in lung cells, we assessed the relations between 4-HNE-modified protein levels, FEV(1), gamma-GCS, and TGF-beta(1). 4-HNE-modified protein levels were elevated in airway and alveolar epithelial cells, endothelial cells, and neutrophils in subjects with COPD, compared with the levels in subjects without COPD ($p < 0.01$). We also observed a significant inverse correlation between the levels of 4-HNE adducts in alveolar epithelium, airway endothelium, and neutrophils and FEV(1) ($p < 0.05$) and a positive correlation between 4-HNE adducts and TGF-beta(1) protein and mRNA as well as gamma-GCS mRNA levels in airway and alveolar epithelium ($p < 0.01$). The elevated levels of 4-HNE may play a role in the signaling events in lung inflammation leading to the imbalance of the expression of both proinflammatory mediators and protective antioxidant genes in COPD.

PMID: 12186826 [PubMed - indexed for MEDLINE]

4: J Cell Physiol 2002 May;191(2):227-36

Malondialdehyde binding to proteins dramatically alters fibroblast functions.

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The regulation of cell metabolism by the surrounding environment is deeply altered by the posttranslational nonenzymatic modifications of extracellular proteins that occur throughout lifespan in vivo and modify their structural and functional properties. Among them are protein adducts formed by components generated from oxidative processes, such as malondialdehyde (MDA). We have investigated here the effects of MDA-binding to proteins on cultured fibroblast functions. Type I collagen and/or serum proteins were incubated with 0-100 mM MDA for 3 h before use in fibroblast cultures. In tridimensional lattice cultures, MDA-treated collagen inhibited the contracting activity of fibroblasts. A similar inhibition of lattice contraction was reproduced by the addition of MDA-treated serum to the culture medium. In monolayer cultures, the addition of MDA-modified serum proteins completely inhibited fibroblast multiplication without effect on initial adhesion steps. MDA-modified proteins decreased the proliferative capacities of cells, strongly altered cell cycle progression by blocking passage to G2/M phases, and induced apoptotic features in fibroblasts. Our results show, for the first time, that MDA-modified proteins are potentially as deleterious as free MDA, and could be involved in aging as well as in degenerative complications of diseases with increased oxidative stress such as diabetes mellitus or atherosclerosis.

PMID: 12064466 [PubMed - indexed for MEDLINE]

5: J Biol Chem 2002 Aug 2;277(31):27919-26

Thiolation of protein-bound carcinogenic aldehyde. An electrophilic acrolein-lysine adduct that covalently binds to thiols.

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Acrolein, a representative carcinogenic aldehyde that could be ubiquitously generated in biological systems under oxidative stress, shows facile reactivity with the epsilon-amino group of lysine to form N(epsilon)-(3-formyl-3,4-dehydropiperidino)lysine (FDP-lysine) as the major product (Uchida, K., Kanematsu, M., Morimitsu, Y., Osawa, T., Noguchi, N., and Niki, E. (1998) J. Biol. Chem. 273, 16058-16066). In the present study, we determined the electrophilic potential of FDP-lysine and established a novel mechanism of protein thiolation in which the FDP-lysine generated in the acrolein-modified protein reacts with sulfhydryl groups to form thioether adducts. When a sulfhydryl enzyme, glyceraldehyde-3-phosphate dehydrogenase, was incubated with acrolein-modified bovine serum albumin in sodium phosphate buffer (pH 7.2) at 37 degrees C, a significant loss of sulfhydryl groups, which was accompanied by the loss of enzyme activity and the formation of high molecular mass protein species (>200 kDa), was observed. The FDP-lysine adduct generated in the acrolein-modified protein was suggested to represent a thiol-reactive electrophile based on the following observations. (i) N(alpha)-acetyl-FDP-lysine, prepared from the reaction of N(alpha)-acetyl lysine with acrolein, was covalently bound to glyceraldehyde-3-phosphate dehydrogenase. (ii) The FDP-lysine derivative reacted with glutathione to form a GSH conjugate. (iii) The acrolein-modified bovine serum albumin significantly reacted with GSH to form a glutathiolated protein. Furthermore, the observation that the glutathiolated acrolein-modified protein showed decreased immunoreactivity with an anti-FDP-lysine monoclonal antibody suggested that the FDP-lysine residues in the acrolein-modified protein served as the binding site of GSH. These data suggest that thiolation of the protein-bound acrolein may be involved in redox alteration under oxidative stress, whereby oxidative stress generates the increased production of acrolein and its protein adducts that further potentiate oxidative stress via the depletion of GSH in the cells.

PMID: 12032148 [PubMed - indexed for MEDLINE]

PM3006739587

6: Toxicology 2002 May 1;173(3):193-201

Protein adducts of malondialdehyde and 4-hydroxynonenal in livers of iron loaded rats: quantitation and localization.

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Pathophysiological mechanisms for hepatocellular injury, fibrosis and/or cirrhosis in hepatic iron overload are poorly understood. An increase in intracellular transit pool of iron can catalyze peroxidation of lipids to produce reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). Covalent binding of such lipid aldehydes with proteins may cause impairment in cellular function and integrity. This investigation was focused on quantitative determination of MDA and HNE-protein adducts, and to establish a correlation between iron deposition and formation and localization of MDA and HNE-protein adducts, using immunohistochemistry. To achieve iron overload, male SD rats were fed a 2.5% carbonyl iron-supplemented diet for six weeks, while control animals received standard diet. Total iron as well as low molecular weight chelatable iron (LMWC-Fe) in the hepatic tissue of rats fed the iron supplemented diet increased significantly (approximately 14- and approximately 15-fold, respectively). Quantitative ELISA for MDA-and HNE-protein adducts showed remarkable increases of 186 and 149%, respectively, in the liver homogenates of rats fed the iron-supplemented diet. Sections of liver stained for iron showed striking iron deposits in periportal (zone 1) hepatocytes, which was less dramatic in midzonal (zone 2) cells. Livers from iron-loaded rats showed strong, diffuse staining for both MDA and HNE adducts, which was highly pronounced in centrilobular (zone 3) hepatocytes, but was also evident in midzonal cells (zone 2). The demonstration of greater formation of both MDA and HNE-protein adducts provides evidence of iron-catalyzed lipid peroxidation in vivo. Although in this model of iron overload there was no evidence of tissue injury, our results provide an account of some of the initiating factors or early molecular events in hepatocellular damage that may lead to the pathological manifestations seen in chronic iron overload.

PMID: 11960672 [PubMed - indexed for MEDLINE]

7: Early Hum Dev 2002 Apr;67(1-2):19-28

Immunological detection of 4-hydroxynonenal protein adducts in developing pontine and Purkinje neurons and in karyorrhexis in pontosubicular neuronal necrosis.

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Four-hydroxynonenal (HNE) has been proposed as an important marker of radical-induced lipid peroxidation. The principal objective of this study was to assess the occurrence of lipid peroxidation in normal perinatal brain and brains with one form of pontosubicular neuronal necrosis (PSN). Immunochemical studies using an antibody against HNE-modified protein were performed in controls aged from 20 weeks of gestation to 64 years, and patients with PSN. Immunohistochemical study showed developmental and aging changes of positive staining in Purkinje cells and pontine neurons (27 weeks-7 months, 50 and 64 years). In addition, karyorrhectic cells in pontine nuclei with PSN were positively stained. Immunoblotting revealed that a 75-kDa protein, which is speculated to be mitochondrial complex-1 protein, was the most intensely expressed among multiple immunoreactive proteins. Our results identified the presence of oxidative stress in the perinatal neuron, and this oxidative stress may contribute to some forms of karyorrhectic death.

PMID: 11893432 [PubMed - indexed for MEDLINE]

8: Free Radic Biol Med 2002 Feb 15;32(4):303-8

Role of malondialdehyde-acetaldehyde adducts in liver injury(1,2).

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Malondialdehyde and acetaldehyde react together with proteins in a synergistic manner and form hybrid protein adducts, designated as MAA adducts. MAA-protein adducts are composed of two major products whose structures and mechanism of formation have been elucidated. MAA adduct formation, especially in the liver, has been demonstrated in vivo during ethanol consumption. These protein adducts are capable of inducing a potent immune response, resulting in the generation of antibodies against both MAA epitopes, as well as against epitopes on the carrier protein. Chronic ethanol administration to rats results in significant circulating antibody titers against MAA-adducted proteins, and high anti-MAA titers have been associated with the severity of liver damage in humans with alcoholic liver disease. In vitro exposure of liver endothelial or hepatic stellate cells to MAA adducts induces a proinflammatory and profibrogenic response in these cells. Thus, during excessive ethanol consumption, ethanol oxidation and ethanol-induced oxidative stress result in the formation of acetaldehyde and malondialdehyde, respectively. These aldehydes can react together synergistically with proteins and generate MAA adducts, which are very immunogenic and possess proinflammatory and profibrogenic properties. By virtue of these potentially toxic effects, MAA adducts may play an important role in the pathogenesis of alcoholic liver injury.

PMID: 11841919 [PubMed - in process]

9: J Biol Chem 2002 Feb 15;277(7):4644-8

High molecular weight neurofilament proteins are physiological substrates of adduction by the lipid peroxidation product hydroxynonenal.

Wataya T, Nunomura A, Smith MA, Siedlak SL, Harris PL, Shimohama S, Szweda LI, Kaminski MA, Avila J, Price DL, Cleveland DW, Sayre LM, Perry G.

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Protein adducts of the lipid peroxidation product trans-4-hydroxy-2-nonenal (HNE) are features of oxidative damage in neuronal cell bodies in Alzheimer's disease but are also seen in axons of normal as well as diseased individuals. In this study, focusing on the axons of the mouse sciatic nerve, we found that HNE adducts characterize axons of mice from birth to senility. Immunoblots of axonal proteins showed that HNE adducts are only detected in neurofilament heavy subunit (NFH) and, to a lesser extent, neurofilament medium subunit (NFM), both lysine-rich proteins, consistent with the adducts being limited to lysine residues. In vitro, HNE treatment of permeabilized sciatic nerve showed the same specificity, i.e. NFH and NFM are the only proteins that reacted with HNE, providing they are phosphorylated. Quantitative immunoblot analysis of two strains of mice ages 1-33 months showed that the levels of HNE adducts on NFH are consistent throughout life. Additionally, mice transgenic for human superoxide dismutase-1 with G85R mutation show no difference in HNE adduction to NFH compared with controls. Taken together, these studies indicate that HNE adduction to NFH is physiological, and its constancy from birth to senility as well as its dependence on phosphorylation argues that NFH and NFM modification may play a role in protecting the membrane-rich axon from toxic aldehydes resulting from oxidative damage.

PMID: 11733539 [PubMed - indexed for MEDLINE]

10: Free Radic Biol Med 2001 Dec 15;31(12):1533-8

Distribution of ethanol-induced protein adducts in vivo: relationship to tissue injury.

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epitopes

Generation of oxygen free radicals and reactive aldehydes as a result of excessive ethanol consumption has been well established. Recent studies in human alcoholics and in experimental animal models have indicated that acetaldehyde, the first metabolite of ethanol, and the aldehydic products of lipid peroxidation can bind to proteins in tissues forming stable adducts. The demonstration of such adducts in zone 3 hepatocytes in alcoholics with an early phase of histological liver damage indicates that adduct formation may have an important role in the sequence of events leading to alcoholic liver disease. There may be interference with cellular functions, stimulation of fibrogenesis, and immunological responses. Autoantibodies towards distinct types of adducts have been shown to be associated with the severity of liver disease in alcoholic patients. High fat diet and/or iron supplementation combined with ethanol may increase the amount of aldehyde-derived epitopes and promote fibrogenesis in the liver. Recently, ethanol-derived protein modifications have also been found from other tissues exposed to ethanol and acetaldehyde, including rat brain after lifelong ethanol administration, pancreas, and rat muscle. Elevated adduct levels also occur in erythrocytes of alcoholics, which may be related to ethanol-induced morphological aberrations in hematopoiesis.

Publication Types:
Review
Review, Tutorial

PMID: 11744326 [PubMed - indexed for MEDLINE]

PM3006739592

11: Alcohol Clin Exp Res 2001 Nov;25(11):1648-53

Assays for acetaldehyde-derived adducts in blood proteins based on antibodies against acetaldehyde/lipoprotein condensates.

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BACKGROUND: Acetaldehyde-derived protein condensates (adducts) have been suggested as promising biological markers of alcohol abuse because they represent actual metabolites of ethanol. However, the detection of such condensates in vivo has been hampered by a lack of sensitive and specific methods. METHODS: To develop new approaches for the detection of acetaldehyde adducts, we have raised antibodies against condensates with acetaldehyde and lipoproteins, which have previously been shown to be readily modified by acetaldehyde in vitro. The characteristics of these antibodies were compared with those raised against bovine serum albumin/acetaldehyde adduct and against other types of lipoprotein modifications, as induced by malondialdehyde, oxidation, and acetylation. RESULTS: The antibodies raised against low-density lipoprotein (LDL)/acetaldehyde, very low density lipoprotein (VLDL)/acetaldehyde, and bovine serum albumin/acetaldehyde all reacted with protein adducts generated at physiologically relevant concentrations of acetaldehyde in vitro, whereas the antibodies raised against malondialdehyde/LDL, oxidized LDL, or acetylated LDL were not found to cross-react with the acetaldehyde-derived adducts. In assays for acetaldehyde adducts from erythrocyte and serum proteins of patients with excessive ethanol consumption (n = 32) and healthy control individuals (n = 22), the antibody prepared against the acetaldehyde/VLDL condensate was found to provide the most effective detection of acetaldehyde adducts in vivo. CONCLUSIONS: Current data indicate that acetaldehyde generates immunogenic adducts with lipoproteins in vivo. Antibodies raised against the VLDL/acetaldehyde may provide a basis for new diagnostic assays to examine excessive alcohol consumption.

PMID: 11707639 [PubMed - indexed for MEDLINE]

12: Arch Dermatol Res 2001 Jul;293(7):363-7

Immunohistochemical detection of lipid peroxidation products, protein-bound acrolein and 4-hydroxynonenal protein adducts, in actinic elastosis of photodamaged skin.

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Acrolein and 4-hydroxy-2-nonenal (HNE) are both byproducts of a lipid peroxidation reaction. Actinic elastosis in photodamaged skin of aged individuals is characterized by the accumulation of fragmented elastic fibers in the sun-exposed areas. To study whether a lipid peroxidation reaction is involved in the accumulation of altered elastic fibers in actinic elastosis, skin specimens obtained from sun-damaged areas were immunohistochemically examined using the antibodies against acrolein and HNE. Both antibodies were found to react with the accumulations of elastic material. Double immunofluorescence labeling demonstrated that acrolein/elastin and HNE/elastin were colocalized in the actinic elastosis. Western blot analysis showed that the polypeptide with a molecular weight of 62 kDa reacted with anti-acrolein, anti-HNE and anti-elastin antibodies. The results suggest that acrolein and HNE may be associated with actinic elastosis.

PMID: 11550810 [PubMed - indexed for MEDLINE]

13: J Biol Chem 2001 Jun 29;276(26):23903-13 got it !

Endogenous formation of protein adducts with carcinogenic aldehydes: implications for oxidative stress.

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In the present study, we characterize the covalent modification of a protein by crotonaldehyde, a representative carcinogenic aldehyde, and describe the endogenous production of this aldehyde in vivo. The crotonaldehyde preferentially reacted with the lysine and histidine residues of bovine serum albumin and generated a protein-linked carbonyl derivative. Upon incubation with the histidine and lysine derivatives, crotonaldehyde predominantly generated beta-substituted butanal adducts of histidine and lysine and N(epsilon)-(2,5-dimethyl-3-formyl-3,4-dehydropiperidino)lysine (dimethyl-FDP-lysine) as the putative carbonyl derivatives generated in the crotonaldehyde-modified protein. To verify the endogenous formation of crotonaldehyde in vivo, we raised the monoclonal antibody (mAb82D3) against the crotonaldehyde-modified protein and found that it cross-reacted with the protein-bound 2-alkenals, such as crotonaldehyde, 2-pentenal, and 2-hexenal. The anti-2-alkenal antibody recognized multiple crotonaldehyde-lysine adducts, including dimethyl-FDP-lysine and an unknown product, which showed the greatest immunoreactivity with the antibody. On the basis of the chemical and spectroscopic evidence, the major antigenic product was determined to be a novel Schiff base-derived crotonaldehyde-lysine adduct, N(epsilon)-(5-ethyl-2-methylpyridinium)lysine (EMP-lysine). It was found that the lysine residues that had disappeared in the protein treated with crotonaldehyde were partially recovered by EMP-lysine. The presence of immunoreactive materials with mAb82D3 in vivo was demonstrated in the kidney of rats exposed to the renal carcinogen, ferric nitrilotriacetate. In addition, the observations that the metal-catalyzed oxidation of polyunsaturated fatty acids in the presence of proteins resulted in an increase in the antigenicity of the protein indicated that lipid peroxidation represents a potential pathway for the formation of crotonaldehyde/2-alkenals in vivo. These data suggest that the formation of carcinogenic aldehydes during lipid peroxidation may be causally involved in the pathophysiological effects associated with oxidative stress.

PMID: 11283024 [PubMed - indexed for MEDLINE]

14. Toxicology 2001 Mar 7;160(1-3):207-17

Cigarette smoke impairs neutrophil respiratory burst activation by aldehyde-induced thiol modifications.

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Exposure to airborne pollutants such as tobacco smoke is associated with increased activation of inflammatory-immune processes and is thought to contribute to the incidence of respiratory tract disease. We hypothesized that cigarette smoke (CS) could synergize with activated inflammatory/immune cells to cause oxidative injury or result in the formation of unique reactive oxidants. Isolated human neutrophils were exposed to gas-phase CS, and the production of nitrating and chlorinating oxidants following neutrophil stimulation was monitored using the substrate 4-hydroxyphenylacetate (HPA). Stimulation of neutrophils in the presence of CS resulted in a reduced oxidation and chlorination of HPA, suggesting inhibition of NADPH oxidase or myeloperoxidase (MPO), the two major enzymes involved in inflammatory oxidant formation. Peroxidase assays demonstrated that neutrophil MPO activity was not significantly affected after CS-exposure, leaving the NADPH oxidase as a likely target. The inhibition of neutrophil oxidant formation was found to coincide with depletion of cellular GSH, and a similar modification of critical cysteine residues, such as those in NADPH oxidase components, might be involved in reduced respiratory burst activity. As alpha,beta-unsaturated aldehydes such as acrolein have been implicated in thiol modifications by CS, we exposed neutrophils to acrolein prior to stimulation, and observed inhibition of NADPH oxidase activation in relation to GSH depletion. Additionally, translocation of the cytosolic components of NADPH oxidase to the membrane, a necessary requirement for enzyme activation, was inhibited. Protein adducts of acrolein (or related aldehydes) could be detected in several neutrophil proteins, including NADPH oxidase components, following neutrophil exposure to either CS or acrolein. Alterations in neutrophil function by exposure to (environmental) tobacco smoke may affect inflammatory/infectious conditions and thereby contribute to tobacco-related disease.

PMID: 11246141 [PubMed - indexed for MEDLINE]

15. Arch Biochem Biophys 2000 Dec 1;384(1):81-7

Cytochrome P450 2E1 (CYP2E1)-dependent production of a 37-kDa acetaldehyde-protein adduct in the rat liver.

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Ethanol-inducible cytochrome P450 2E1 (CYP2E1) has been shown to be involved in the metabolism of both ethanol and acetaldehyde. Acetaldehyde, produced from ethanol metabolism, is highly reactive and can form various protein adducts. In this study, we investigated the role of CYP2E1 in the production of a 37-kDa acetaldehyde-protein adduct. Rats were pairfed an isocaloric control or an alcohol liquid diet with and without cotreatment of YH439, an inhibitor of CYP2E1 gene transcription, for 4 weeks. The soluble proteins from rat livers of each group were separated on SDS-polyacrylamide gels followed by immunoblot analysis using specific antibodies against the 37-kDa protein acetaldehyde adduct. In addition, catalytic activities of the enzymes involved in alcohol and acetaldehyde metabolism were measured and compared with the adduct level. Immunoblot analysis revealed that the 37-kDa adduct, absent in the pair-fed control, was evident in alcohol-fed rats but markedly reduced by YH439 treatment. Immunohistochemical analysis also showed that the 37-kDa adduct is predominantly localized in the pericentral region of the liver where CYP2E1 protein is mainly expressed. This staining disappeared in the pericentral region after YH439 treatment. The levels of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase isozymes were unchanged after YH439 treatment. However, the level of the 37-kDa protein adduct positively correlated with the hepatic content of P4502E1. These data indicate that the 37-kDa adduct could be produced by CYP2E1-mediated ethanol metabolism in addition to the ADH-dependent formation.

PMID: 11147839 [PubMed - indexed for MEDLINE]

16: J Hepatol 2000 Dec;33(6):926-32

Covalent adducts of proteins with acetaldehyde in the liver as a result of acetaldehyde administration in drinking water.

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BACKGROUND/AIMS: Acetaldehyde, the first metabolic product of ethanol, has been suggested to be responsible for several adverse effects of ethanol through its ability to form covalent adducts with proteins and cellular constituents. It has recently been suggested that acetaldehyde derived from microbial ethanol oxidation in the gut could also contribute to the effects of ethanol in the liver. The present work aimed to examine whether modification of proteins by acetaldehyde occurs in rat liver as a result of acetaldehyde administration in drinking water. METHODS: Rats were fed with either 0.7% acetaldehyde (n=10) or water (n=10) for 11 weeks. At the end of the feeding period, liver specimens were processed for immunohistochemistry for protein adducts with acetaldehyde and for hepatic cell type-specific protein markers. RESULTS: Mild fatty change was found in the liver of the acetaldehyde-treated animals but not in the control animals. Immunohistochemical stainings for acetaldehyde adducts revealed intensive positive staining for acetaldehyde adducts in eight (80%) of the animals fed with acetaldehyde. The adducts were predominantly perivenular, although positive staining also occurred along the sinusoids and in the periportal area. Double immunofluorescence staining experiments revealed that hepatocytes were the primary targets of acetaldehyde adduct deposition, although stellate cells and Kupffer cells also showed weak positive reactions. CONCLUSIONS: The present data indicate that acetaldehyde-protein adducts are formed in the liver of animals following acetaldehyde administration in drinking water, which may contribute to the hepatotoxicity of extrahepatic acetaldehyde. These findings should be implicated in studies on the extrahepatic pathways of ethanol oxidation.

PMID: 11131454 [PubMed - indexed for MEDLINE]

17: J Hepatol 2000 Dec;33(6):893-901

Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases.

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BACKGROUND/AIMS: Interaction between CYP2E1, ethanol metabolites, and enhanced lipid peroxidation is linked to the pathogenesis of alcoholic liver disease. This study was conducted to compare the expression of various cytochrome enzymes and the appearance of aldehyde adducts in humans. METHODS: Acetaldehyde- and lipid peroxidation-derived protein adducts and CYP2A6, 2E1, and 3A4/5 were examined immunohistochemically from liver specimens of 12 alcohol abusers with either mild (n=7) or severe (n=5) liver disease, and from nine non-drinking patients with non-alcoholic steatosis (n=4), or hepatitis (n=5). RESULTS: Ethanol-inducible CYP2E1 was present in all alcoholic livers. While CYP2A6 in zone 3 hepatocytes was also abundant in the alcoholic patients with various degrees of liver disease, CYP3A415 was most prominent in alcoholic cirrhosis. The sites of CYP2E1 and CYP2A6 immunoreactivity co-localized with fatty deposits, and with the sites of acetaldehyde and lipid peroxidation-derived protein adducts. The CYP enzymes were also abundant in the centrilobular hepatocytes of patients with fatty liver due to obesity or diabetes. CONCLUSIONS: Alcohol-induced liver damage is associated with a generalized induction of CYP2A6, CYP2E1 and CYP3A4 and generation of acetaldehyde and lipid peroxidation-derived protein-aldehyde adducts. However, CYP induction also occurred in patients with non-alcoholic steatosis.

PMID: 11131450 [PubMed - indexed for MEDLINE]

18: Methods Mol Biol 2000;99:25-34

Quantitation of 4-hydroxynonenal protein adducts.

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Publication Types:
Review
Review, Tutorial

PMID: 10909074 [PubMed - indexed for MEDLINE]

19: Toxicol Appl Pharmacol 1999 Nov 15;161(1):23-33

4-Hydroxynonenal and malondialdehyde hepatic protein adducts in rats treated with carbon tetrachloride: immunochemical detection and lobular localization.

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The metabolism of CCl(4) initiates the peroxidation of polyunsaturated fatty acids producing alpha,beta-unsaturated aldehydes, such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). The facile reactivity of these electrophilic aldehydic products suggests they play a role in the toxicity of compounds like CCl(4). To determine the rate at which CCl(4)-initiated lipid peroxidation results in the formation of 4-HNE and/or MDA hepatic protein adducts, rats were given an intragastric dose of CCl(4) (1.0 ml/kg) and euthanized 0-72 h after administration. Rabbit polyclonal antisera directed toward 4-HNE- or MDA-protein epitopes were employed in immuno-histochemical and immuno-precipitation/Western analyses to detect 4-HNE and MDA-protein adducts in paraffin-embedded liver sections and liver homogenates. As early as 6 h post CCl(4) exposure, 4-HNE and MDA adducts were detected immuno-histochemically in hepatocytes localized to zone 2 of the hepatic acinus. Liver injury was progressive to 24 h as lipid peroxidation and hepatocellular necrosis increased. The hallmark of CCl(4) hepatotoxicity, zone 3 necrosis, was observed 24 h after CCl(4) administration and immuno-positive hepatocytes were observed in zone 2 as well as zone 3. Immuno-positive cells were no longer visible by 36 to 72 h post CCl(4) administration. From 6 to 48 h after CCl(4) administration, at least four adducted proteins were immuno-precipitated from liver homogenates with the anti-MDA or anti-4HNE serum, which corresponded to molecular weights of 80, 150, 205, and greater than 205 kDa. These results demonstrate that 4-HNE and MDA alkylate specific hepatic proteins in a time-dependent manner, which appears to be associated with hepatocellular injury following CCl(4) exposure.

PMID: 10558920 [PubMed - indexed for MEDLINE]

20: Hepatology 1999 Oct;30(4):1011-7

Induction of cytochrome P450 enzymes and generation of protein-aldehyde adducts are associated with sex-dependent sensitivity to alcohol-induced liver disease in micropigs.

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To assess possible links between ethanol-induced oxidant stress, expression of hepatic cytochrome P450 (CYP) enzymes, and sex steroid status, we used immunohistochemical methods to compare the generation of protein adducts of acetaldehyde (AA), malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE) with the amounts of CYP2E1, CYP2A, and CYP3A in the livers of castrated and noncastrated male micropigs fed ethanol for 12 months. In castrated micropigs, ethanol feeding resulted in accumulation of fat, hepatocellular necrosis, inflammation, and centrilobular fibrosis, whereas only minimal histopathology was observed in their noncastrated counterparts. CYP2A and CYP3A were more prominent in the castrated animals than in the noncastrated micropigs. Ethanol feeding increased the hepatic content of all CYP forms. The most significant increases occurred in CYP2E1 and CYP3A in the noncastrated animals and in CYP2E1 and CYP2A in the castrated animals. Ethanol-fed castrated animals also showed the greatest abundance of perivenular adducts of AA, MDA, and HNE. In the noncastrated ethanol-fed micropigs a low expression of each CYP form was associated with scant evidence of aldehyde-protein adducts. Significant correlations emerged between the levels of different CYP forms, protein adducts, and plasma levels of sex steroids. The present findings indicate that the generation of protein-aldehyde adducts is associated with the induction of several cytochrome enzymes in a sex steroid-dependent manner. It appears that the premature, juvenile, metabolic phenotype, as induced by castration, favors liver damage. The present findings should be implicated in studies on the gender differences on the adverse effects of ethanol in the liver.

PMID: 10498654 [PubMed - indexed for MEDLINE]

21: Kidney Int Suppl 1999 Jul;71:S54-6

Increased carbonyl modification by lipids and carbohydrates in diabetic nephropathy.

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BACKGROUND: In diabetic nephropathy (DN), possible mediators of untoward effects of hyperglycemia include the advanced glycation end products (AGEs). Indeed, an AGE, carboxymethyllysine (CML), accumulates in expanded mesangial matrix and nodular lesions. An advanced lipoxidation end product (ALE), malondialdehyde-lysine (MDA-lysine), generated on proteins during lipid peroxidation also accumulates in these lesions. As both ALEs and AGEs are formed by carbonyl amine chemistry between protein and carbonyl compounds derived from autoxidation of lipids and carbohydrates, their colocalization suggests an increased carbonyl modification of proteins. METHODS: To address this hypothesis, human diabetic renal tissues were examined to characterize carbonyl modification of proteins by lipids and carbohydrates: (a) ALEs, MDA-lysine and 4-hydroxynonenal (HNE) protein adduct, derived from lipids, and (b) AGEs, pentosidine and CML, derived from carbohydrates. Furthermore, to elucidate the biological effect of carbonyl modification on primary cultured human and rat mesangial cells, the intracellular protein phosphorylation was examined in the presence of various kinds of carbonyl compounds. RESULTS: The ALE and AGE adducts examined were identified in expanded mesangial matrix and nodular lesions. The exposure of cultured mesangial cells to carbonyl compounds resulted in phosphorylation of tyrosine residues of a number of intracellular proteins. CONCLUSIONS: These data suggest a broad derangement in nonenzymatic biochemistry involving both lipids and carbohydrates exists in diabetic glomerular lesions ("carbonyl stress").

PMID: 10412738 [PubMed - indexed for MEDLINE]

22: Front Biosci 1999 Jun 1;4:D506-13

Aldehyde-protein adducts in the liver as a result of ethanol-induced oxidative stress.

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A number of systems that generate oxygen free radicals and reactive aldehydic species are activated by excessive ethanol consumption. Recent studies from human alcoholics and from experimental animals have indicated that acetaldehyde and aldehydic products of lipid peroxidation, which are generated in such processes, can bind to proteins forming stable adducts. Adduct formation may lead to several adverse consequences, such as interference with protein function, stimulation of fibrogenesis, and induction of immune responses. The presence of protein adducts in the centrilobular region of the liver in alcohol abusers with an early phase of histological liver damage indicates that adduct formation is one of the key events in the pathogenesis of alcoholic liver disease. Dietary supplementation with fat and/or iron strikingly increases the amount of aldehyde-derived epitopes in the liver together with promotion of fibrogenesis.

Publication Types:
Review
Review, Tutorial

PMID: 10352137 [PubMed - indexed for MEDLINE]

23: Alcohol Clin Exp Res 1999 Apr;23(4):657-63

Conversion of acetaldehyde-protein adduct epitopes from a nonreduced to a reduced phenotype by antigen processing cells.

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Many investigators have suggested that an immune reaction to acetaldehyde-protein adducts may be involved in the development and/or progression of alcohol liver disease. The most often reported acetaldehyde adduct is the reduced adduct prepared in vitro in the presence of strong reducing agents. However, the production of this adduct in vivo has been difficult to prove. Nevertheless, the detection of serum antibodies to this reduced adduct following alcohol exposure in animals and humans has been used to support the formation of this adduct in vivo. We have recently observed that when acetaldehyde-protein adducts prepared under nonreducing conditions are used to immunize animals, antibody to the reduced protein adduct is detected. Therefore, it was the purpose of this study to demonstrate that nonreduced (NR) adduct epitopes can be modified by intact cells to express reduced (R) adduct epitopes. This was accomplished using the monoclonal antibody RT1.1 that has been previously characterized by this laboratory and has been shown to recognize only R and not NR acetaldehyde adducts. In these studies, Balb/c mice were injected intraperitoneally (500 microg/animal) with either keyhole limpet hemocyanin (KLH)-NR or KLH-R adducted proteins. Immunization with KLH-NR produced significant amounts of antibodies that recognized both NR and R epitopes. In contrast, immunization with KLH-R produced antibodies to only R and not NR epitopes. Isolated peritoneal macrophages from nonimmunized mice were incubated in vitro with either KLH-NR, KLH-R, or unmodified KLH proteins, and the cell surface expression of the reduced epitope (RT1.1) and the activated macrophage marker (MAC-3) determined by double immunofluorescent staining. Activated macrophages incubated with KLH-NR expressed the R adduct on 11.5% of the cells, compared with 3.8% following incubation with unmodified KLH, and 19.4% following incubation with KLH-R. These data suggest that the NR adduct and/or the carrier protein are modified by peritoneal macrophages in vivo and present an epitope that is detected as a reduced adduct (RT1.1 positive). These observations may explain the presence of circulating antibodies to the reduced adduct that has been reported in human and animal studies.

PMID: 10235301 [PubMed - indexed for MEDLINE]

24. J Biol Chem 1999 May 7;274(19):13139-46

Identification of extremely reactive gamma-ketoaldehydes (isolevuglandins) as products of the isoprostane pathway and characterization of their lysyl protein adducts.

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Isoprostanes are prostaglandin-like compounds produced by non-enzymatic peroxidation of arachidonic acid. The cyclooxygenase-derived endoperoxide, prostaglandin H2, can undergo rearrangement to highly reactive gamma-ketoaldehyde secoprostanoids (levuglandin E2 and D2). We explored whether isoprostane endoperoxide intermediates also rearrange to levuglandin-like compounds (isolevuglandins). Formation of a series of isolevuglandins during oxidation of arachidonic acid in vitro was established utilizing a number of mass spectrometric analyses. However, these compounds could not be detected in free form in protein-containing biological systems, which we hypothesized was due to extremely rapid adduction to amines. This was supported by the finding that >60% of levuglandin E2 adducted to albumin within 20 s, whereas approximately 50% of 4-hydroxynonenal still remained unadducted after 1 h. By utilizing electrospray tandem mass spectrometry, we established that these compounds form oxidized pyrrole adducts (lactams and hydroxylactams) with lysine. Formation of isolevuglandin-lysine adducts on apolipoprotein B was readily detected during oxidation of low density lipoprotein following enzymatic digestion of the protein to single amino acids. These studies identify a novel series of extremely reactive products of the isoprostane pathway that rapidly form covalent adducts with lysine residues on proteins. This provides the basis to explore the formation of isolevuglandins in vivo to investigate the potential biological ramifications of their formation in settings of oxidant injury.

PMID: 10224068 [PubMed - indexed for MEDLINE]

25. Arch Biochem Biophys 1999 May 1;365(1):101-12

Age-dependent renal accumulation of 4-hydroxy-2-nonenal (HNE)-modified proteins following parenteral administration of ferric nitrilotriacetate commensurate with its differential toxicity: implications for the involvement of HNE-protein adducts in oxidative stress and carcinogenesis.

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In this study, we show that the toxicity of ferric nitrilotriacetate (Fe-NTA) can be correlated with the tissue accumulation of 4-hydroxy-2-nonenal (HNE)-modified protein adducts. It is observed that the toxic manifestations of Fe-NTA gradually increase with the increasing age of animals. A dose of Fe-NTA which produces almost 100% mortality in aged rats causes 70% mortality in adults, 30% in pups, 20% in litters, and less than 10% in neonates. The age-dependent increase in its toxicity is also evident from the data of renal microsomal lipid peroxidation and hydrogen peroxide generation. No significant difference in the generation of H_2O_2 and induction of renal microsomal lipid peroxidation between saline- and Fe-NTA-treated neonates, litters, and pups could be observed. However, in adult rats, a significant increase in both of the parameters was observed which was even greater in aged rats. On the contrary, renal glutathione levels in these animals show an inverse relationship with the oxidant generation. In neonates, litters, and pups the maximum decrease of glutathione was up to 22%, whereas in adult and aged rats, the depletion was more than 60% of their respective saline-treated controls. Parallel to this data, blood urea nitrogen and creatinine, the indicators of renal damage, show a significant increase in Fe-NTA-treated adult and aged rats only, whereas no significant alterations were observed in other groups. Similarly, the magnitude of ODC induction and [3H]thymidine incorporation was much higher in aged and adult rats in comparison to other groups of animals after Fe-NTA treatment. Additionally, the immunohistochemical localization studies show a significant increase in HNE-modified protein adducts in kidney of adult and aged rats, whereas no significant staining was observed in other groups. A similar increase in the level of protein carbonyls has also been observed with the increasing age of rats. These data suggest that the toxicity of Fe-NTA increases with the increasing age of rats and correlates with the accumulation of HNE-modified protein adducts. It may also be speculated that Fe-NTA-mediated renal toxicity leading to carcinogenesis may be related to the tissue accumulation of HNE-modified protein adducts. However, further studies are needed to establish a definite role of HNE-modified proteins in Fe-NTA-mediated carcinogenesis.

PMID: 10222044 [PubMed - indexed for MEDLINE]

26: J Am Soc Nephrol 1999 Apr;10(4):822-32

Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions.

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Advanced glycation end products (AGE) include a variety of protein adducts whose accumulation has been implicated in tissue damage associated with diabetic nephropathy (DN). It was recently demonstrated that among AGE, glycoxidation products, whose formation is closely linked to oxidation, such as carboxymethyllysine (CML) and pentosidine, accumulate in expanded mesangial matrix and nodular lesions in DN, in colocalization with malondialdehyde-lysine (MDA-lysine), a lipoxidation product, whereas pyrraline, another AGE structure whose deposition is rather independent from oxidative stress, was not found within diabetic glomeruli. Because CML, pentosidine, and MDA-lysine are all formed under oxidative stress by carbonyl amine chemistry between protein amino group and carbonyl compounds, their colocalization suggests a local oxidative stress and increased protein carbonyl modification in diabetic glomerular lesions. To address this hypothesis, human renal tissues from patients with DN or IgA nephropathy were examined with specific antibodies to characterize most, if not all, carbonyl modifications of proteins by autoxidation products of carbohydrates, lipids, and amino acids: CML (derived from carbohydrates, lipids, and amino acid), pentosidine (derived from carbohydrates), MDA-lysine (derived from lipids), 4-hydroxynonenal-protein adduct (derived from lipids), and acrolein-protein adduct (derived from lipids and amino acid). All of the protein adducts were identified in expanded mesangial matrix and nodular lesions in DN. In IgA nephropathy, another primary glomerular disease leading to end-stage renal failure, despite positive staining for MDA-lysine and 4-hydroxynonenal-protein adduct in the expanded mesangial area, CML, pentosidine, and acrolein-protein adduct immunoreactivities were only faint in glomeruli. These data suggest a broad derangement in nonenzymatic biochemistry in diabetic glomerular lesions, and implicate an increased local oxidative stress and carbonyl modification of proteins in diabetic glomerular tissue damage ("carbonyl stress").

Publication Types:
Clinical Trial
Controlled Clinical Trial

PMID: 10203367 [PubMed - indexed for MEDLINE]

PM3006739607

27: Alcohol Clin Exp Res 1998 Dec;22(9):2118-24

Early alcoholic liver injury: formation of protein adducts with acetaldehyde and lipid peroxidation products, and expression of CYP2E1 and CYP3A.

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The formation of protein adducts with reactive aldehydes resulting from ethanol metabolism and lipid peroxidation has been suggested to play a role in the pathogenesis of alcoholic liver injury. To gain further insight on the contribution of such aldehydes in alcoholic liver disease, we have compared the appearance of acetaldehyde, malondialdehyde, and 4-hydroxynonenal adducts with the expression of cytochrome P-450IIE1, and cytochrome P-4503A enzymes in the liver of rats fed alcohol with a high-fat diet for 2 to 4 weeks according to the Tsukamoto-French procedure and in control rats (high-fat liquid diet or no treatment). Urine alcohol and serum aminotransferase levels were recorded, and the liver pathology was scored from 0 to 10 according to the presence of steatosis, inflammation, necrosis, and fibrosis. The ethanol treatment resulted in the accumulation of fat, mild necrosis and inflammation, and a mean liver pathology score of 3 (range: 1 to 5). Liver specimens from the ethanol-fed animals with early alcohol-induced liver injury were found to contain perivenular, hepatocellular acetaldehyde adducts. Malondialdehyde and 4-hydroxynonenal adducts were also present showing a more diffuse staining pattern with occasional sinusoidal reactions. In the control animals, a faint positive reaction for the hydroxynonenal adduct occurred in some of the animals fed the high fat diet, whereas no specific staining was observed in the livers from the animals receiving no treatment. Expression of both CYP2E1 and CYP3A correlated with the amount of protein adducts in the liver of alcohol-treated rats. Distinct CYP2E1-positive immunohistochemistry was seen in 3 of 7 of the ethanol-fed animals. In 5 of 7 of the ethanol-fed animals, the staining intensities for CYP3A markedly exceeded those obtained from the controls. The present findings indicate that acetaldehyde and lipid peroxidation-derived adducts are generated in the early phase of alcohol-induced liver disease. The formation of protein adducts appears to be accompanied by induction of both CYP2E1 and CYP3A.

PMID: 9884160 [PubMed - indexed for MEDLINE]

28: Atherosclerosis 1998 Nov;141(1):107-16

Association of malondialdehyde-acetaldehyde (MAA) adducted proteins with atherosclerotic-induced vascular inflammatory injury.

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Atherosclerosis is a vascular injury characterized by elevated tissue levels of tumor necrosis factor-alpha (TNF-alpha), increased expression of endothelial cell adhesion molecules, and vascular wall inflammatory cell infiltration. Foam cells are associated with atherosclerotic plaque material, and low density lipoprotein (LDL) is a lipid component of foam cells. Malondialdehyde (MDA) is

an oxidative product of unsaturated fatty acids and is also present in atherosclerotic lesions. MDA-modified (adducted) proteins, including MDA-modified LDL, are present in atherosclerotic human vascular tissue. Acetaldehyde (AA) is the major metabolic product of ethanol oxidation. Both MDA and AA are highly reactive aldehydes and will combine with proteins to produce an antigenically distinct protein adduct, termed the MAA adduct. This study demonstrates that proteins modified in the presence of high concentrations of MDA can produce MAA-modified proteins in vitro. In addition, MAA adducted proteins are capable of inducing rat heart endothelial cell cultures (rHEC) to produce and release TNF-alpha, and cause rHEC upregulation of endothelial adhesion molecule expression, including ICAM-1. These adhesion molecules are required for circulating inflammatory cells to adhere to endothelium which allows inflammatory cell tissue infiltration. Additionally, MAA modified proteins were defected in human atherosclerotic aortic vascular tissue but not in normal aortic tissue. Since atherosclerosis is associated with an inflammatory vascular injury characterized by elevated tissue TNF-alpha concentrations and inflammatory cell infiltration, these data suggest that MAA-adducted proteins may be formed in atherosclerotic plaque material and may be involved in the inflammatory reaction that occurs in atherosclerosis. These data further suggest that previous studies demonstrating MDA modified protein in atherosclerotic plaque may in fact have MAA modified proteins associated with them.

PMID: 9863543 [PubMed - indexed for MEDLINE]

29: Atherosclerosis 1998 Oct;140(2):357-63

Endogenous formaldehyde as a potential factor of vulnerability of atherosclerosis: involvement of semicarbazide-sensitive amine oxidase-mediated methylamine turnover.

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The mouse is known to be highly resistant to atherosclerosis. However, some inbred mouse strains are vulnerable to atherosclerosis when they are fed a high-cholesterol, high-fat diet. Increased deamination of methylamine (MA) and the subsequent production of formaldehyde has been recently shown to be a potential risk factor of atherosclerosis. In the present study semicarbazide-sensitive amine oxidase (SSAO)-mediated MA turnover in C57BL/6 mouse, a strain very susceptible to atherosclerosis, has been assessed in comparison to a moderate, i.e. BALB/c, and resistant, i.e. CD1, mouse strains. Kidney and aorta SSAO activities were found to be significantly increased in C57BL/6 in comparison to BALB/c and CD1 mice. A significant increase of urinary MA and formaldehyde were detected in C57BL/6. [14C]MA following intravenous injection would be quickly metabolized by SSAO. The labeled formaldehyde product would cross link with proteins. C57BL/6 exhibits significantly higher labeled protein adducts than BALB/c and CD1 in response to [14C]MA. The results indicated that mice vulnerable to atherosclerosis possess an increased SSAO-mediated MA turnover. The increase of production of formaldehyde, possibly other aldehydes, may induce endothelial injury or be chronically involved in protein cross-linking and subsequent angiopathy.

PMID: 9862279 [PubMed - indexed for MEDLINE]

30: Biochem Pharmacol 1998 Nov 15;56(10):1371-9

U-101033E (2,4-diaminopyrrolopyrimidine), a potent inhibitor of membrane lipid peroxidation as assessed by the production of 4-hydroxynonenal, malondialdehyde, and 4-hydroxynonenal--protein adducts.

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4-Hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) are major lipid peroxidation products generated by free radical attack on membranes and appear to contribute to the cytotoxic effects of oxidative stress by a mechanism involving adduct formation with cellular proteins. In the present studies, we investigated the relationship between lipid peroxidation and eventual inactivation of plasma membrane proteins using a model system consisting of purified red blood cell membranes and Fe2+/EDTA. Using this system, we also analyzed the ability of a novel antioxidant, U-101033E (2,4-diaminopyrrolopyrimidine), to inhibit lipid peroxidation and associated protein damage. Our results demonstrated that significant levels of MDA and 4-HNE are generated in this model system, and that both aldehydes are capable of cross-linking membrane proteins. In addition, we used a monoclonal antibody to demonstrate the presence of 4-HNE-protein adducts in this system. The generation of 4-HNE-protein adducts closely paralleled the time course of lipid peroxidation and membrane protein cross-linking, suggesting that 4-HNE may contribute to membrane protein cross-linking. Analysis of U-101033E in this system showed that this antioxidant inhibited lipid peroxidation, prevented the appearance of 4-HNE-protein adducts, and strongly reduced membrane protein cross-linking, with an EC50 of 0.5 microM. We also show that these antioxidant effects were not due to the scavenging of superoxide anion. Thus, these studies demonstrate the potential usefulness of U-101033E for treating certain disease processes where lipid peroxidation plays a role in disease pathogenesis.

PMID: 9825737 [PubMed - indexed for MEDLINE]

31: FEBS Lett 1998 Oct 16;437(1-2):24-8

Generation of protein carbonyls by glycoxidation and lipoxidation reactions with autoxidation products of ascorbic acid and polyunsaturated fatty acids.

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Accumulation of carbonyl derivatives of proteins (protein carbonyl) is taken as a biomarker of oxidative protein damage in aging and in various diseases. We detected protein carbonyls in situ in human diabetic arteriosclerotic tissues and characterized the formation of protein carbonyls. Protein carbonyls were identified in the thickened intima of arterial walls and co-localized with

protein adducts formed by carbonyl amine chemistry between protein and carbonyl compounds derived from autoxidation of carbohydrates, lipids, and ascorbate, i.e. advanced glycation end products or glycoxidation products, such as carboxymethyllysine (CML) and pentosidine, and lipoxidation products, such as malondialdehyde (MDA) and 4-hydroxy-nonenal (HNE). In vitro incubation of proteins with ascorbic acid accelerated the production of protein carbonyls as well as CML and pentosidine, and incubation with arachidonate accelerated the production of protein carbonyls as well as CML, MDA, and HNE. By contrast, incubation of proteins with glucose resulted in the production of CML and pentosidine, but not protein carbonyls. Schiff base inhibitors, (+/-)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylace tanilide and aminoguanidine, inhibited the production of protein carbonyls after incubation with ascorbate and arachidonate. The present study suggests that ascorbate and polyunsaturated fatty acids, but not glucose, represent potential sources of protein carbonyls, and that both the glycoxidation and lipoxidation reactions contribute to protein carbonyl formation in aging and various diseases.

PMID: 9804165 [PubMed - indexed for MEDLINE]

32: J Neuropathol Exp Neurol 1998 May;57(5):415-25

Distribution of reducible 4-hydroxynonenal adduct immunoreactivity in Alzheimer disease is associated with APOE genotype.

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Two major risk factors for late-onset familial and sporadic Alzheimer disease (AD), a leading cause of dementia worldwide, are increasing age and inheritance of the epsilon4 allele of the apolipoprotein E gene (APOE4). Several isoform-specific effects of apoE have been proposed; however, the mechanisms by which apoE isoforms influence the pathogenesis of AD are unknown. Also associated with AD is increased lipid peroxidation in the regions of the brain most damaged by disease. 4-hydroxynonenal (HNE), the most potent neurotoxic product of lipid peroxidation, is thought to be deleterious to cells through reactions with protein nucleophiles. We tested the hypothesis that accumulation of the most common forms of HNE-protein adducts, borohydride-reducible adducts, is associated with AD and examined whether there was a relationship to APOE. Our results demonstrated that reducible HNE adducts were increased in the hippocampus, entorhinal cortex, and temporal cortex of patients with AD. Furthermore, our data showed that the pattern of reducible HNE adduct accumulation was related to APOE genotype; AD patients homozygous for APOE4 had pyramidal neuron cytoplasmic accumulation of reducible HNE adducts, while AD APOE3 homozygotes had both pyramidal neuron and astrocyte accumulation of reducible HNE adducts. This is in contrast to our previous observations that a distinct HNE protein adduct, the pyrrole adduct, accumulates on neurofibrillary tangles in AD patients. We conclude that APOE genotype influences the cellular distribution of increased reducible HNE adduct accumulation in AD.

PMID: 9596412 [PubMed - indexed for MEDLINE]

33: Biochem J 1998 Apr 1;331 (Pt 1):185-91

Oxidation of high-density lipoprotein HDL3 leads to exposure of apo-AI and apo-AII epitopes and to formation of aldehyde protein adducts, and influences binding of oxidized low-density lipoprotein to type I and type III collagen in vitro1.

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The changes in the immunological properties of apolipoprotein AI (apo-AI) and AII (apo-AII) during the oxidation of the high-density lipoprotein HDL3 and its influence on the binding of heavily oxidized low-density lipoprotein (LDL) to type I and III collagen were investigated. Oxidation of HDL3 or Eu3+-labelled HDL3 was performed with CuSO4, varying the time of oxidation. Oxidation of HDL3 resulted in an increase in lipid hydroperoxides and enhanced the negative charge of this lipoprotein. Immunological studies with a solid-phase sandwich immunoassay revealed a strong increase in binding of Eu3+-labelled HDL3 to polyclonal antibodies against apo-AI and apo-AII within the first 4 h of oxidation. Neo-epitopes were also formed by interaction of the apolipoproteins with degradation products from the lipid peroxidation of polyunsaturated fatty acids, as evidenced by an immunoreaction of oxidized Eu3+-labelled HDL3 with antibodies to 4-hydroxynonenal (4-HNE)- and malondialdehyde (MDA)-protein adducts. Western blot analysis of oxidized HDL3 samples showed, as well as apo-AI and apo-AII bands, larger aggregated apolipoproteins, occurring after 0.5-2.5 h of oxidation. These aggregates were recognized by antibodies to apo-AI and apo-AII as well as by antibodies to 4-HNE- and MDA-protein adducts. Furthermore the original apo-AI monomers and apo-AII dimers decreased during the oxidation. The ability of native and oxidized HDL3 to prevent the binding of Eu3+-labelled 24 h-oxidized LDL to collagen on microtitration plates was estimated. Interestingly, 2 h-oxidized HDL3 competed most with the binding of 24 h-oxidized LDL on collagen type I and type III, followed by native HDL3. However, 24 h-oxidized HDL3 was a weaker competitor. Thus oxidative modification of HDL3 strongly alters the immunological properties of this lipoprotein and its binding affinity for collagen.

PMID: 9512478 [PubMed - indexed for MEDLINE]

34: Chem Res Toxicol 1998 Feb;11(2):136-42

Stable acetaldehyde--protein adducts as biomarkers of alcohol exposure.

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The consumption of alcoholic beverages has been associated with increased risks of a number of chronic disorders including cancers. It is still not clear whether ethyl alcohol or other components such as metabolites are directly involved in the carcinogenic process or whether the effects are due to the modulation of metabolism of other carcinogens. At present, there is no good biomarker of alcohol intake, particularly at low or moderate levels of

consumption. A number of studies have shown the ability of the major metabolite acetaldehyde to react with proteins in vitro to give stable and unstable adducts. The interaction of acetaldehyde with model peptides, which correspond to N-terminal globin sequences, was studied. The major stable adduct was identified by mass spectrometry and NMR as a diastereoisomeric mixture of imidazolidinones. This is believed to be formed by reaction and cyclization of the initial Schiff base adduct with the N-terminal valine. Incubation of human globin with acetaldehyde (0-2 mM) yielded products which were identified as the N-terminal adducts by electrospray ionization mass spectrometry (ESI-MS) of proteolytic digests. The specificity and sensitivity of the analysis was improved by the use of on-line HPLC-ESI-MS. Tryptic digests of the modified globin which contained both the N-terminal acetaldehyde adducts of alpha-globin (heptapeptide) and beta-globin (octapeptide) were resolved. These results suggest that analysis of stable imidazolidinone adducts is a promising approach to estimation of alcohol exposure.

PMID: 9511905 [PubMed - indexed for MEDLINE]

35: Am J Physiol 1998 Jan;274(1 Pt 1):L8-16

Potential involvement of 4-hydroxynonenal in the response of human lung cells to ozone.

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Ozone is a photochemically generated pollutant that can cause acute pulmonary inflammation and induce cellular injury and may contribute to the development or exacerbation of chronic lung diseases. Despite much research, the mechanisms of ozone- and oxidant-induced cellular injury are still uncertain. Ozone and secondary free radicals have been reported to cause the formation of aldehydes in biological fluids. One of the most toxic aldehydes formed during oxidant-induced lipid peroxidation is 4-hydroxynonenal (HNE). HNE reacts primarily with Cys, Lys, and His amino acids, altering protein function and forming protein adducts. The purpose of this study was to determine whether HNE could account for the acute effects of ozone on lung cells. Human subjects were exposed to 0.4 parts/million ozone or air for 1 h with exercise (each subject served as his/her own control). Six hours after ozone exposure, cells obtained by airway lavage were examined for apoptotic cell injury, and cells from bronchoalveolar lavage were examined for apoptosis, presence of HNE adducts, and expression of stress proteins. Significant apoptosis was evident in airway lung cells after ozone exposure. Western analysis demonstrated an increase in a 32-kDa HNE protein adduct and a number of stress proteins, viz., 72-kDa heat shock protein and ferritin, in alveolar macrophages (AM) after ozone exposure. All of these effects could be replicated by in vitro exposure of AM to HNE. Consequently, the in vitro results and demonstration of HNE protein adducts after ozone exposure are consistent with a potential role for HNE in the cellular toxic effects of ozone.

PMID: 9458795 [PubMed - indexed for MEDLINE]

36: Chem Res Toxicol 1997 Sep;10(9):978-86

Epitope characterization of malondialdehyde-acetaldehyde adducts using an enzyme-linked immunosorbent assay.

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Malondialdehyde (MDA) and acetaldehyde react together with proteins in a synergistic manner and form hybrid protein adducts, designated as MAA adducts. In a previous study, a polyclonal antibody specific for MAA-protein adducts was used in an immunoassay to detect the presence of MAA adducts in livers of ethanol-fed rats. In the present study, the specific epitope recognized by the antibody was defined and the chemistry of MAA adduct formation was further characterized. When several synthetic analogs were tested for their ability to inhibit antibody binding in a competitive ELISA, the results indicated that the major determinant of antibody binding was a highly fluorescent cyclic adduct composed of two molecules of MDA and one of acetaldehyde. The structure of this adduct was shown to be a 4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde derivative of an amino group of a protein. Examination of MAA adduct formation with a variety of proteins indicated that in addition to this specific fluorescent adduct, MAA adducts were also comprised of other nonfluorescent products. The amount of fluorescent epitopes present on a given protein was the major determinant of antibody binding as assessed in a competitive ELISA, although the efficiency of inhibition of antibody binding by these fluorescent epitopes on MAA-adducted proteins varied depending upon the particular protein. However, when these MAA-adducted proteins were hydrolyzed with Pronase, the concentration of these modified proteins necessary to achieve 50% inhibition of antibody binding in a competitive ELISA fell into a much narrower range of values, indicating that protein hydrolysis equalized the accessibility of the antibody to bind the epitope on these various derivatized proteins. In summary, a cyclic fluorescent adduct of defined structure has been identified as the epitope recognized by our MAA adduct antibody. In addition to this specific adduct, MAA adducts are also comprised of other nonfluorescent products.

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37: Toxicol Appl Pharmacol 1996 Dec;141(2):416-24

4-hydroxy-2-nonenal-protein adducts and apoptosis in murine lung cells after acute ozone exposure.

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Ozone is a photochemically generated pollutant that can cause acute pulmonary inflammation and induce cellular injury and may contribute to the development or exacerbation of chronic lung diseases. Despite extensive investigation, the mechanisms of ozone and oxidant-induced cellular injury are still uncertain. Ozone has been reported to cause the formation of aldehydes in biological fluids that could explain many of the cellular effects caused by ozone. One of the most

toxic aldehydes formed during oxidant-induced lipid peroxidation is 4-hydroxy-2-nonenal (HNE). HNE reacts primarily with Cys and secondarily with Lys and His amino acids, altering protein function and forming protein adducts that can be detected using specific adducts. In this study, we investigated whether ozone could cause the formation of HNE by assaying for HNE-protein adducts in cells isolated by lung lavage from C3H/HeJ mice exposed to 2.0 and 0.25 ppm ozone for 3 hr. Since oxidative stress and HNE have been shown to cause apoptosis we also examined the lung lavage cells for evidence of apoptosis following ozone exposure. Using a specific polyclonal antibody against HNE-amino acid adducts, two principle HNE-protein adducts were detected by Western analysis in cells obtained after ozone exposure at approximately 86-90 and 32 kDa. In addition to cell necrosis, apoptosis of lung cells was significant 3 hr after ozone exposure as detected using a Cell Death ELISA procedure and confirmed with DNA ladder and morphological analysis. The apoptotic cell injury peaked at 6 hr postexposure and decreased by 24 hr. Taken together, these results demonstrate that HNE is formed in vivo following ozone exposure and HNE appears to form specific protein adducts in lung cells. Furthermore, ozone can cause lung cell injury by an apoptotic mechanism in addition to a necrotic mechanism. Since HNE is toxic to cells and is formed as a result of ozone exposure, it may contribute to the lung cell injury following ozone exposure.

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38: Toxicol Appl Pharmacol 1996 Jul;139(1):135-43

4-Hydroxynonenal-induced cell death in murine alveolar macrophages.

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Oxidative stress is known to cause apoptosis in many cell types, yet the mechanism of oxidative stress-induced apoptosis is not clear. Oxidative stress has been described to cause peroxidation of polyunsaturated fatty acids. 4-Hydroxynonenal (HNE) is a diffusible product of lipid peroxidation and has been shown to be toxic to cells. In this study, the effects of HNE on isolated alveolar macrophages (AM) from two murine strains (C3H/HeJ and C57BL/6J) were examined. HNE induced the formation of protein adducts in AMs from both strains of mice in a dose-dependent manner, and the amounts of HNE-protein adducts formed in cells from both strains were very similar. In the HNE dose range from 1 to 100 microM, AMs from both strains had very little necrosis as shown by trypan blue staining. However, AMs from both C3H/HeJ and C57BL/6J mice had extensive apoptosis at 100 microM HNE, but little or no apoptosis at 25 microM HNE. Furthermore, AMs from C57BL/6J mice had significant apoptosis at 50 microM HNE while AMs from C3H/HeJ mice had no significant apoptosis at this dose. At low doses of HNE (10 to 25 microM), there was induction of heme oxygenase 1. The data indicated that HNE induces apoptosis in murine macrophages, and cells from different strains of mice have different sensitivities to the HNE-induced apoptosis. The cause of the difference in susceptibility is not known, but it is possible that different stress response and/or apoptosis-regulating proteins may be in part responsible. Our observation that a product of lipid peroxidation causes apoptosis suggested that it might be a mediator for oxidative stress-induced apoptosis.

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39: Biochemistry 1996 Jun 18;35(24):8075-83

N (epsilon)-(carboxymethyl)lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction.

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Long-term incubation of proteins with glucose leads to the formation of advanced glycation end products (AGE). Recent immunological studies have suggested the potential role of AGE in atherosclerosis, aging, and diabetic complications. We previously prepared a monoclonal (6D12) as well as a polyclonal anti-AGE antibody and proposed the presence of a common AGE structure(s) that may act as a major immunochemical epitope [Horiuchi, S., Araki, N., & Morino, Y. (1991) J. Biol. Chem. 266, 7329-7332]. The purpose of the present study was to determine the major epitope. Amino acid analysis of AGE-proteins indicated that N(epsilon)-(carboxymethyl)lysine (CML) was a major modified lysine residue. Immunologic studies demonstrated the positive reaction of 6D12 not only to all CML-modified proteins tested, but also to BSA modified with several aldehydes known to generate a CML-protein adduct, and a linear correlation between the CML contents of CML-BSA and their immunoreactivity to 6D12 up to approximately 8 mol/mol of protein. Further experiments with CML analogs revealed that the epitope of 6D12 is a CML-protein adduct with an important carbonyl group. In contrast to 6D12, our polyclonal anti-AGE antibody showed a significant but much weaker immunoreactivity to CML-BSA, suggesting that the polyclonal antibody contains two populations, one reactive to CML (CML-PA) and the other unreactive to CML (Non-CML-PA). Non-CML-PA separated from CML-PA by CML-BSA affinity chromatography did not react with all CML-modified preparations, but retained its property to react commonly with AGE preparations obtained from proteins, lysine derivatives, and monoaminocarboxylic acids. Therefore, it is clear that a CML-protein adduct is a major immunological epitope in AGE structures, but there still exist other major epitope(s) expressed commonly in AGE-proteins.

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40: Proc Natl Acad Sci U S A 1996 Apr 2;93(7):2696-701

Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease.

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There is growing evidence that oxidative stress and mitochondrial respiratory failure with attendant decrease in energy output are implicated in nigral neuronal death in Parkinson disease (PD). It is not known, however, which cellular elements (neurons or glial cells) are major targets of oxygen-mediated damage. 4-Hydroxy-2-nonenal (HNE) was shown earlier to react with proteins to

form stable adducts that can be used as markers of oxidative stress-induced cellular damage. We report here results of immunochemical studies using polyclonal antibodies directed against HNE-protein conjugates to label the site of oxidative damage in control subjects (ages 18-99 years) and seven patients that died of PD (ages 57-78 years). All the nigral melanized neurons in one of the midbrain sections were counted and classified into three groups according to the intensity of immunostaining for HNE-modified proteins--i.e., no staining, weak staining, and intensely positive staining. On average, 58% of nigral neurons were positively stained for HNE-modified proteins in PD; in contrast only 9% of nigral neurons were positive in the control subjects; the difference was statistically significant (Mann-Whitney U test; $P < 0.01$). In contrast to the substantia nigra, the oculomotor neurons in the same midbrain sections showed no or only weak staining for HNE-modified proteins in both PD and control subjects; young control subjects did not show any immunostaining; however, aged control subjects showed weak staining in the oculomotor nucleus, suggesting age-related accumulation of HNE-modified proteins in the neuron. Our results indicate the presence of oxidative stress within nigral neurons in PD, and this oxidative stress may contribute to nigral cell death.

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41: Alcohol Alcohol 1995 May;30(3):373-8

Antibodies made against a formaldehyde-protein adduct cross react with an acetaldehyde-protein adduct. Implications for the origin of antibodies in human serum which recognize acetaldehyde-protein adducts.

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Acetaldehyde, the major metabolite of ethanol, reacts with lysine and other free amino groups on proteins to form acetaldehyde-protein adducts. The presence of antibodies which recognize such acetaldehyde-protein adducts in sera from alcoholics has been attributed to an immune response to such adducts. Complicating this conclusion is the finding that sera from non-alcoholic control subjects also contain antibodies which recognize acetaldehyde-protein adducts. In the current research we sought to determine whether antibodies which recognize epitopes formed by the reaction of a protein with acetaldehyde can be formed in response to a protein modified with a structurally related protein adduct. We modified lysine residues on apolipoprotein (apo) B-100 with acetaldehyde and formaldehyde under reducing conditions, to form epsilon-N-methyl- and epsilon-N-ethyl-lysine residues, and with acetic anhydride to form epsilon-N-acetyl-lysine residues, and made antibodies against these modified proteins in guinea-pigs. In ELISA assays antibodies made against methylated apoB-100 (Me-apoB) cross-reacted effectively with ethylated apoB-100 (Et-apoB), while antibodies made against acetic anhydride-modified apoB-100 did not cross-react. We conclude that methyl-lysine shares one or more immunoreactive epitopes with ethyl-lysine, and that antibodies which recognize acetaldehyde-modified proteins can be formed in response to formaldehyde-modified proteins. We demonstrate that sera from both alcoholics and non-drinkers contain antibodies which recognize Me-apoB and Et-apoB and that the titres of these antibodies are comparable. These data raise the possibility that some human serum antibodies which recognize acetaldehyde-modified protein epitopes may have been made against formaldehyde-modified protein epitopes.(ABSTRACT TRUNCATED AT 250 WORDS)

42: Toxicol Lett 1994 Mar;71(1):27-37

Rapid plasma clearance of albumin-acrolein adduct in rats.

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Protein adducts are used as markers of chemical exposure. Determination of the clearance rate of these adducts from the blood circulation will provide the time frame for their measurement. Radioactive albumin was prepared biosynthetically by repeated intraperitoneal injections of L-[4,5-3H]lysine to a rat. After an affinity purification, an aliquot of this native [3H-lysine]albumin was adducted with 5 mM acrolein. Both the native albumin (A-treated group) and the albumin-acrolein adduct (AAA-treated group) were intravenously injected to separate groups of rats, and the clearance of radioactivity from the plasma was measured as a function of time. At the end of the experiment (33 h after the injection), radioactivity in the whole plasma, and in homogenates of liver, kidney and spleen and their trichloroacetic acid(TCA)-soluble and -insoluble fractions in both A- and AAA-treated groups, was measured. The results, at the initial 11 h after the injection, showed that the radioactivity was cleared from the circulating plasma more rapidly in the AAA-treated group (32% of the injected radioactivity remained) than the A-treated group (52%). At 33 h after the injection, 22% of the injected radioactivity remained in the plasma in the AAA-treated group as compared to 32% in the A-treated group. The whole homogenates of liver and kidney and their corresponding TCA-soluble fractions showed higher radioactivity in the AAA-treated group as compared to the A-treated group. However, the TCA-insoluble fractions from livers and kidneys of the AAA-treated group showed lower radioactivity as compared to the A-treated group. These results indicated that the albumin-acrolein adduct was removed more rapidly from the circulation than the native albumin, and degraded more rapidly by the liver and kidney. There was no preferential removal or degradation of the adducted albumin by the spleen.

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43: Alcohol Clin Exp Res 1994 Feb;18(1):164-71

Detection of reduced acetaldehyde protein adducts using a unique monoclonal antibody.

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Acetaldehyde (AA), the major product of alcohol metabolism, has been shown to bind to proteins in vivo and form chemical adducts. These AA-protein adducts have been shown to alter protein structure and function and may result in tissue damage. Recent reports have shown that polyclonal antibodies can be produced that recognize proteins modified in vitro with AA in the presence of sodium cyanoborohydride (NaCNBH3), a strong reducing (R) agent. Antibodies prepared in this way have been shown to recognize proteins in the livers of rats fed alcohol chronically. Because multiple AA-protein adducts can be recognized by polyclonal antisera, and a variety of adducts may be formed in vitro or in vivo, this study was designed to develop monoclonal antibodies specific for proteins modified by AA. In addition, adducts formed under R conditions are probably chemically different than those formed under nonreducing (NR) conditions, and monoclonal antibodies may provide the specificity required to distinguish these chemical differences. Balb/c mice were immunized with bovine brain tubulin that was modified by treatment with 5 mM AA for 7 days under NR conditions. Sera from immunized animals were tested for antibody activity to the immunogen (protein-NR) and for cross-reactivity to protein-R and unmodified protein. Although the highest serum antibody titers were seen toward the NR adduct, antibodies to the R adduct were also detected. This activity difference was independent of the carrier protein, because NR and R bovine serum albumin, keyhole limpet hemocyanin, and actin also gave similar results when used as the adducted protein.(ABSTRACT TRUNCATED AT 250 WORDS)

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44: Methods Enzymol 1994;233:371-80

Quantitation of 4-hydroxynonenal protein adducts.

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PMID: 8015472 [PubMed - indexed for MEDLINE]

45: J Hepatol 1993 Nov;19(3):385-92

Acetaldehyde-protein adducts, but not lactate and pyruvate, stimulate gene transcription of collagen and fibronectin in hepatic fat-storing cells.

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Hepatic fibrosis is an important morphological feature of alcohol-induced liver injury. We previously reported that acetaldehyde, but not ethanol can stimulate type I collagen and fibronectin synthesis in cultures of rat fat-storing cells (FSC) by increasing transcription of the specific genes. The effect of lactate and pyruvate was studied on collagen I, III, fibronectin accumulation by cultured rat FSCs and it was investigated whether acetaldehyde could increase procollagen I and fibronectin gene transcription through the formation of protein adducts. Lactate and pyruvate (5, 15 and 25 mmol/l) did not significantly affect collagen I, III and fibronectin production by cultured FSCs. Pyridoxal-phosphate and p-hydroxymecuribenzoate (inhibitors of acetaldehyde-protein adduct formation) blocked the stimulatory effect of acetaldehyde on procollagen I and fibronectin gene transcription. These data suggest that ethanol may act as a liver fibrogenic factor through acetaldehyde, its immediate metabolite, whereas lactate does not seem to play a role. Acetaldehyde might stimulate gene transcription of extracellular matrix components by liver FSCs through the formation of adducts with proteins.

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46: Life Sci 1988;43(20):1633-41

Formation of cyanomethyl derivatives of basic amino acids and proteins with components in cigarette smoke.

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A reaction of the basic amino acids, lysine and arginine, with components of cigarette smoke has been observed. The adducts produced have been identified as cyanomethyl derivatives. Both formaldehyde and cyanide, which are known to be present in cigarette smoke, are involved in the reaction with the primary amino group. The reaction is time-dependent and can be enhanced by an increase of temperature or by incubation under alkaline conditions. Cyanomethyl adduct formation was found to be increased when smoke from cigarettes with higher tar and nicotine content was used. When proteins, such as bovine serum albumin, trypsin inhibitors or crude rat lung proteins were incubated with the cigarette smoke solution, new protein adducts with increased pI values were produced which are separable from the original proteins by gel isoelectric focussing. Radioisotopically labelled cyanide can be irreversibly linked to protein and the linkage is enhanced in the presence of formaldehyde.

PMID: 3143035 [PubMed - indexed for MEDLINE]